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UV-B Irradiation Does Not Promote Flowering in *Arabidopsis* Despite Increased *FT* Expression

Ami Takahashi | Yuki Takahashi | Jun Hidema | Mika Teranishi

Graduate School of Life Sciences, Tohoku University, Sendai, Japan

Correspondence: Mika Teranishi (mika.teranishi.d8@tohoku.ac.jp)

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ABSTRACT

Various environmental factors control the plant flowering time. However, the specific effects of ultraviolet (UV)-B radiation on flowering remain unclear. UV-B irradiation delays flowering in *Arabidopsis* during short-day (SD) photoperiods. In contrast, UV-B irradiation causes a variety of flowering phenotypes during long-day (LD) photoperiods, including unchanged, delayed, and accelerated flowering. We hypothesized that variations in UV-B intensity are responsible for the phenotypic changes under LD photoperiods. Therefore, in this study, *Arabidopsis* plants were exposed to two distinct UV-B intensities: a low UV-B intensity that activates UVR8-dependent pathways and high UV-B intensity that activates both UVR8-dependent and -independent pathways. Under LD photoperiods, neither the wild-type (WT) nor the *uvr8* mutant showed any change in flowering time at either UV-B irradiation intensity. Under the SD photoperiod, UV-B irradiation delayed WT flowering. The expression of flowering locus T (*FT*) increased after UV-B irradiation under the LD photoperiod in a UVR8-dependent manner. However, despite the increased expression of *FT*, expression levels of floral meristem identity genes in shoot apical meristem (SAM) were not increased by UV-B irradiation. As UV-B irradiation is known to suppress flowering in SAM in a UVR8-dependent manner, increase in *FT* expression induced by UV-B irradiation possibly antagonized the suppressive effect of UV-B irradiation. Overall, these results suggest that flowering phenotypes do not change with UV-B intensity but with the balance between the inhibitory and promotive effects of UVR8 activated by UV-B irradiation.

1 | Introduction

Flowering is an essential developmental process for the survival of plants. The flowering period is controlled by the surrounding environment and is critical for the plant reproductive success. Light and temperature conditions influence both plant growth and flowering time (Li et al. 2022; Takagi et al. 2023). Seasonal variation in day length is a key factor determining the flowering time (Kinoshita and Richter 2020; Takagi et al. 2023). In addition to light and temperature, abiotic stressors also affect flowering. Drought stress and low nitrogen accelerate flowering, whereas salt stress delays flowering in *Arabidopsis* (Li et al. 2007; Riboni et al. 2013; Sanagi et al. 2021). Therefore, various environmental factors influence plant flowering.

Ultraviolet B (UV-B) radiation (280–315 nm) from sunlight is an important environmental factor. UV-B radiation is absorbed by cellular components including proteins, lipids, and DNA, resulting in cellular damage (Chen et al. 2022). Cyclobutane pyrimidine dimers (CPDs) are a type of DNA damage induced by UV-B irradiation. CPDs are the principal causes of UV-B stress-induced growth inhibition in plants (Landry et al. 1997; Hidema et al. 2007). Growth inhibition caused by UV-B irradiation was significantly increased by deficiency of CPD photolyase, which

is responsible for CPD repair. UV-B radiation not only causes cytotoxicity but also acts as a signal when received by the UV-B photoreceptor UV resistance locus 8 (UVR8) (Kliebenstein et al. 2002). Upon exposure to UV-B radiation, UVR8 is converted from an inactive homodimer to an active monomer (Rizzini et al. 2011). Activated UVR8 binds to the constitutive photomorphogenic 1 (COP1)/suppressor of the phyA-105 (SPA) E3 ubiquitin ligase complex (COP1/SPA) and stabilizes the transcription factors that are targeted by COP1/SPA for degradation (Favory et al. 2009; Cloix et al. 2012). Thus, UVR8 activation by UV-B irradiation leads to changes in gene expression. Genes whose expression is increased by UV-B irradiation via UVR8 include UV-absorbing compounds and CPD photolyase, which help protect plants against UV-B damage or to ameliorate its damaging effects (Brown et al. 2005). UVR8 is activated by very low levels of UV-B that do not cause DNA damage (Brown and Jenkins 2008). Higher doses of UV-B radiation have been shown to activate both UVR8-dependent and UVR8-independent responses, with an estimated threshold of 1 $\mu mol\, m^{-2} s^{-1}$ (Brosché and Strid 2002; Brown and Jenkins 2008).

UV-B radiation has been shown to affect flowering with varying effects depending on the photoperiod. *Arabidopsis* flowering is delayed when exposed to UV-B irradiation during short-day (SD) photoperiods (Arongaus et al. 2018; Dotto et al. 2018). In contrast, the effect of UV-B irradiation on *Arabidopsis* flowering during long-day (LD) photoperiods is unclear, with reports indicating unchanged, delayed, or accelerated flowering (Arongaus et al. 2018; Dotto et al. 2018; Zioutopoulou et al. 2022). Findings from *uvr8* mutants indicate that UVR8 plays a role in this process (Dotto et al. 2018; Zioutopoulou et al. 2022). However, two contradictory results have been reported: one indicates that UV-B irradiation suppresses the expression of flowering locus T (*FT*), a crucial regulator of flowering, in a UVR8-dependent manner, and the other indicates that it enhances the expression of *FT* in a UVR8-dependent manner. Thus, the effects of UV-B irradiation on flowering vary with the day length, and the reasons for these differences are unknown. One possibility is that the UV-B light source and irradiation intensity differed between these reports. Irradiation at an intensity of 2 $W\, m^{-2} s^{-1}$ for 1 h per day delayed flowering (Dotto et al. 2018), while irradiation at an intensity of 0.7 $W\, m^{-2} s^{-1}$ for 16 h per day had no effect on flowering (Arongaus et al. 2018). Irradiation with low fluence UV-B of 0.5 $\mu mol\, m^{-2} s^{-1}$ for 16 h per day accelerated flowering (Zioutopoulou et al. 2022). Comparing light intensities expressed in W $m^{-2} s^{-1}$ and $\mu mol\ m^{-2} s^{-1}$ is not straightforward; nevertheless, the UV-B intensity of 0.5 $\mu mol\, m^{-2} s^{-1}$ utilized by Zioutopoulou et al. (2022) is described as one twentieth of the UV-B intensity of 2 $W\, m^{-2} s^{-1}$ employed by Dotto et al. (2018). These reports suggest that the flowering phenotype may vary with UV-B intensity, with high intensities delaying flowering and low intensities accelerating flowering. However, because of the variations in the spectra of UV-B radiation emitted by different light sources, it is difficult to directly compare the findings of different studies.

In this study, two different UV-B intensities and different irradiation times were used to analyze the effects of UV-B irradiation on flowering. Notably, no changes in flowering time were detected under any of the tested UV-B irradiation conditions under the LD photoperiod. However, under the SD photoperiod, UV-B irradiation delayed the flowering of WT plants, which is consistent with previous reports. *FT* expression increased in a UVR8-dependent manner under all UV-B irradiation conditions only under the LD photoperiod. Although UV-B irradiation elevated *FT* expression, no corresponding increase in the expression levels of floral meristem identity genes in SAM was observed under the LD photoperiod. It is possible that UV-B intensity does not determine the flowering phenotype, but that the flowering phenotype changes according to the balance between the already known inhibitory effect of UV-B irradiation on flowering and the promotive effect of increased *FT* expression.

2 | Materials and Methods

2.1 | Plant Materials and Growth Conditions

Arabidopsis thaliana ecotypes, Landsberg *erecta* (L*er*) and Columbia (Col-0), were used as the wild-type (WT) plants. *uvr8-2* (L*er* background) and *uvr8-6* (Col-0 background; SALK_033468) were used as *uvr8* mutants (Brown et al. 2005; Favory et al. 2009). *uvr2-1* (L*er* background; CS8325) and *phr1* (Col-0 background; CS853162) were used as the CPD photolyase mutants (Landry et al. 1997).

Arabidopsis plants were grown in soil pots at 23°C under LD (16 h light/8 h dark) and SD (8 h light/16 h dark) photoperiods and white fluorescent lamps (approximately 100 $\mu mol\, m^{-2} s^{-1}$, recorded with a data logger, LI-1000; Li-Cor Inc., Lincoln, NE, USA). UV-B irradiation was initiated 8 days after sowing (DAS) after the development of true leaves. Seedlings were irradiated with UV-B bulbs (FL20SE; Toshiba, Tokyo, Japan). A glass filter was placed between the UV-B bulb and plants, reducing the transmission at 300 nm to 50% and blocking wavelengths below 270 nm (Toshiba Glass Co., Shizuoka, Japan). UV-B intensity at the plant level was 0.1 or 0.6 $W\, m^{-2} s^{-1}$ as measured using a data logger (LI-1000; Li-Cor Inc.). Next, spectral irradiance was measured at intervals of 1 nm using a spectroradiometer (USR-45DA; Ushio Inc., Tokyo, Japan) (Figure S1). A photon flux was determined by converting the irradiance of each wavelength using Planck's constant. The photon fluxes between 280 and 315 nm were summed, and total photon fluxes were calculated to be 0.25 and 1.54 $\mu mol\, m^{-2} s^{-1}$ for the irradiance of 0.1 and 0.6 $W\, m^{-2} s^{-1}$, respectively.

When plants were irradiated for 15.5 h per day under the LD photoperiod and 7.5 h per day under the SD photoperiod, plants were irradiated with UV-B for approximately the same duration as the photoperiod, except for 15 min from the beginning and 15 min before the end of the photoperiod. When irradiated for 1 h per day, the irradiation time was 1 h using Zeitgeber time (ZT) 7.5–8.5 and 3.5–4.5 for LD and SD photoperiods, respectively. The daily irradiation dose at an intensity of 0.1 $W\, m^{-2} s^{-1}$ for durations of 1, 7.5, and 15.5 h corresponds to approximately 0.4, 2.7, and 5.6 $kJ\, m^{-2}$, respectively. The daily irradiation dose at an intensity of 0.6 $W\, m^{-2} s^{-1}$ for durations of 1 and 15.5 h corresponds to approximately 2.2 and 33.5 $kJ\, m^{-2}$, respectively. In 1999, average daily UV-B dose in Munich, Germany, was 1.4 $kJ\, m^{-2}$ from September to October and 22 $kJ\, m^{-2}$ from June to August (Kaffarnik et al. 2006). The daily UV-B dose of 33.5 $kJ\, m^{-2}$ exceeds this range, but otherwise, the daily doses

of UV-B irradiation in this experiment ranged from 0.4 to 5.6 kJ m^{-2}, reflecting the levels observed in nature.

2.2 | Measurement of Flowering and Growth

The day of bolting was determined by visual measurement when the stem was elongated and separated from the rosette leaves by a few millimeters. The numbers of rosette and cauline leaves were counted no more than 2 days after the first flower opened. The fresh weight of rosette leaves was determined by measuring the fresh weight of the above-ground parts after removing the inflorescence stems at the base.

2.3 | Sample Collection for Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Samples were collected 10 DAS, 13 DAS, and 17 DAS at ZT15-16, or 29 DAS at ZT7-8 for the LD and SD photoperiods, respectively. The above-ground parts of *Arabidopsis* were separated into rosette leaves and shoot apices containing the shoot apical meristem (SAM) using a needle and stereomicroscope. The rosette leaves were wrapped in aluminum foil, frozen in liquid nitrogen, and stored at −80°C for RNA extraction. The separated shoot apices were frozen in a tube with liquid nitrogen and stored at −80°C for RNA extraction.

2.4 | RNA Extraction and RT-PCR Analysis

Frozen rosette leaves were ground into a powder in liquid nitrogen using a mortar and pestle. A portion of the crushed rosette leaf was transferred to a tube containing 500 μL of TRI reagent (Molecular Research Center, OH, Cincinnati, USA). The rosette leaf was homogenized at 4000 rpm for 1 min using zirconia beads measuring ø1.5 mm using a Micro Smash MS-100R homogenizer (Tomy Seiko Co., Ltd., Tokyo, Japan). Chloroform was added to the tubes, and total RNA was extracted according to the manufacturer's instructions of TRI reagent. cDNA was synthesized using a PrimeScript RT Reagent Kit with gDNA Eraser (Takara Bio Inc., Shiga, Japan). Real-time quantitative RT-PCR was performed using a KAPA SYBR Fast qPCR Kit (NIPPON Genetics Co., Ltd., Tokyo, Japan) with the primers listed in Supplementary Table S1. Data were normalized to *ACTIN* (AT3G18780). For the 10 DAS and 13 DAS samples, the experiment was repeated thrice with three plants per biological replicate. For 17 DAS and 29 DAS samples, the experiment was repeated six times using different plants.

For shoot apices, zirconia beads were added to the tube and homogenized without solution at 4000 rpm for 1 min. TRI reagent was then added, and analyses were performed as described above.

2.5 | Statistical Analyses

Statistical analyses were conducted using the Student's *t*-test with Microsoft Excel 2019 or Tukey's multiple-comparison test with R version 4.0.5.

3 | Results

3.1 | UV-B Irradiation Does Not Alter the Flowering of WT Under the LD Photoperiod

To determine whether flowering time is affected by UV-B intensity, experiments were conducted using the same light source at different UV-B intensities of 0.1 and 0.6 W m^{-2} s^{-1}. UV-B irradiation intensities of 0.1 and 0.6 W m^{-2} s^{-1} are equivalent to 0.25 and 1.54 μmol m^{-2} s^{-1}, respectively. Therefore, intensity of 0.1 W m^{-2} s^{-1} used in this study is weaker than the 0.5 μmol m^{-2} s^{-1} intensity that accelerated flowering in the study of Zioutopoulou et al. (2022).

In previous studies, no difference was reported in growth under the 2 W m^{-2} s^{-1} for 1 h per day UV-B irradiation condition, which delayed flowering, or the 0.5 μmol m^{-2} s^{-1} for 16 h per day UV-B irradiation condition, which accelerated flowering, compared with that in UV-B non-irradiation conditions (Dotto et al. 2018; Zioutopoulou et al. 2022). Therefore, we compared the growth of *Arabidopsis* plants irradiated for 1 h per day within the range of UV-B intensities used in this experiment (Supplementary Figure S2). Under UV-B irradiation in the range of 0.1 to 0.6 W m^{-2} s^{-1} for 1 h per day under LD conditions, no significant difference was observed in the growth of rosette leaves in the WT L*er* and Col-0 ecotypes compared with that in the UV-B nonirradiated control. In contrast, *Arabidopsis* deficient in CPD photolyase, which is responsible for repairing CPD DNA damage induced by UV-B, was comparable to the UV-B nonirradiated control at 0.1 W m^{-2} s^{-1} UV-B intensity for 1 h per day, but growth was significantly inhibited at 0.6 W m^{-2} s^{-1} UV-B intensity for 1 h per day. These results suggest that UV-B intensity of 0.6 W m^{-2} s^{-1} for 1 h per day induces CPD, but UV-B intensity of 0.1 W m^{-2} s^{-1} for 1 h per day does not induce CPD or induces CPD at a level that is repaired by a mechanism other than CPD photolyase.

UVR8 has been reported to be involved in flowering under UV-B irradiation (Arongaus et al. 2018; Dotto et al. 2018; Zioutopoulou et al. 2022). Here, effects of UV-B irradiation on flowering were examined in WT *Arabidopsis* and *uvr8* mutant plants. Representative images of 24 DAS *Arabidopsis* plants exposed to 0.1 W m^{-2} s^{-1} for 15.5 h per day showed that the flowering phenotypes were similar between the WT L*er* ecotype and *uvr8-2* plants as well as between UV-B-irradiated and nonirradiated controls in terms of bolting and flowering (Figure 1a). UV-B irradiation did not affect the numbers of rosette and cauline leaves at the first flower opening in both the WT and *uvr8-2* plants (Figure 1b). However, UV-B irradiation slightly increased the number of days before bolting in WT plants from 19.9 days in the nonirradiated control to 20.5 days in the UV-B-irradiated plants. The number of days before bolting in *uvr8-2* plants showed no significant change after UV-B irradiation. The fresh weight of WT plants did not change, whereas that of *uvr8-2* plants significantly reduced after UV-B irradiation (Figure 1d). Upon repetition of independent experiments, the effects of UV-B irradiation on the flowering phenotype were similar throughout both tests (Table S2). Next, to examine if reducing the UV-B irradiation duration might accelerate flowering, the irradiation duration was set at 1 h. UV-B irradiation did not affect the numbers of rosette and cauline leaves at the first flower opening, nor the number of days before bolting in both the WT and *uvr8-2*

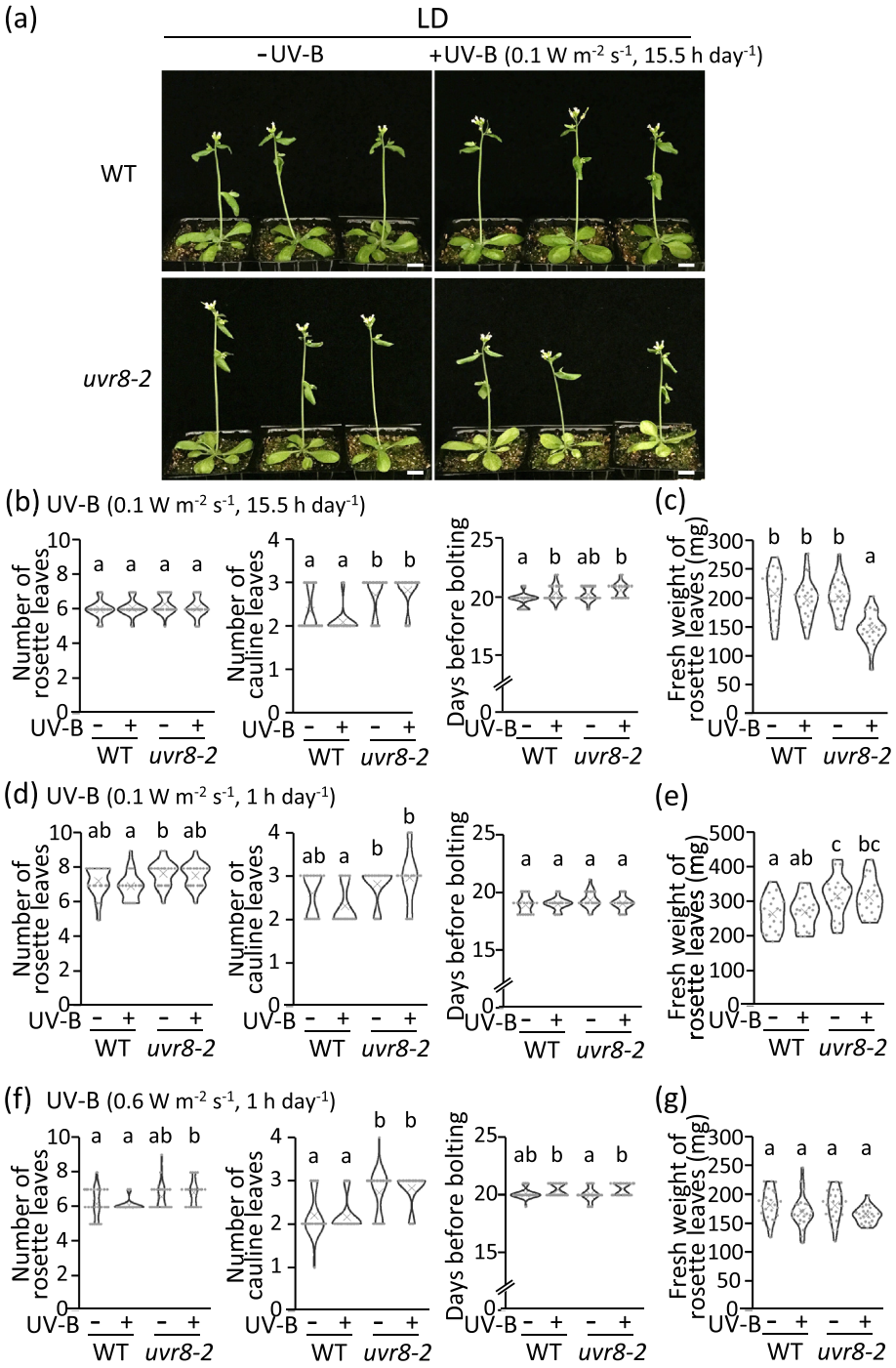

FIGURE 1 | Ultraviolet (UV)-B irradiation does not affect the flowering under the long-day (LD) photoperiod. (a) Representative images of 24-day-old wild-type (WT) and *uvr8-2* plants grown under the LD photoperiod without (−) or with (+) UV-B irradiation at an intensity of 0.1 W m^{-2} s^{-1}. The irradiation time of UV-B was 15.5 h per day. Bars = 1 cm. (b–g) WT and *uvr8-2* plants were grown under the LD photoperiod without (−) or with (+) UV-B irradiation at an intensity of 0.1 W m^{-2} s^{-1} for 15.5 h per day (b, c), 0.1 W m^{-2} s^{-1} for 1 h per day (d, e), or 0.6 W m^{-2} s^{-1} for 1 h per day (f, g). Flowering time was measured by the numbers of rosette and cauline leaves observed when the first flower opened and the number of days before bolting (b, d, f). Fresh weight of rosette leaves was measured when the first flower opened (c, e, g). Data indicate the values of 23–25 plants (n = 23–25) (b, c, f, g) or 19–20 plants (n = 19–20) (d, e). Representative images corresponding to (d, e) and (f, g) are shown in Supplementary Figures S3a and S3c, respectively. Letters on the top of bars indicate the statistical significance determined via Tukey's multiple-comparison test ($p < 0.05$).

plants (Figure 1d and Supplementary Figure S3a). Although the fresh weight of rosette leaves was greater in the *uvr8-2* compared with the WT, no difference was observed with or without UV-B irradiation (Figure 1e). The results indicate that exposure to UV-B intensity of 0.1 W m^{-2} s^{-1} under LD conditions did not significantly affect flowering, regardless of whether the exposure duration was 1 or 15.5 h per day.

Zioutopoulou et al. (2022) reported that irradiation of both L*er* and Col-0 ecotypes with 0.5 μmol m^{-2} s^{-1} UV-B radiation

promotes plant flowering. However, the promoting effect was more pronounced in the Col-0 ecotype in their study, suggesting a difference between ecotypes. Therefore, we performed the analysis with UV-B for 15.5 h per day at an intensity of 0.1 $W m^{-2} s^{-1}$, using the Col-0 ecotype and *uvr8-6*, with Col-0 as the background. We found no differences in the number of rosette leaves, the number of cauline leaves at flowering, and the number of days before bolting between plants with and without UV-B irradiation (Supplementary Figure S4). Under UV-B nonirradiated conditions, *uvr8-6* plants had more rosette leaves and more days to bolt than Col-0 plants; however, no change was observed in the presence or absence of UV-B irradiation.

To examine the effect of elevated UV-B intensity on flowering, UV-B was applied at 0.6 $W m^{-2} s^{-1}$ for 15.5 h per day under LD conditions. As a result, UV-B irradiation significantly inhibited the growth of WT and completely stopped the growth of *uvr8-2* (Supplementary Figure S3b). Consequently, we examined the flowering of plants subjected to UV-B irradiation at an intensity of 0.6 $W m^{-2} s^{-1}$ for 1 h per day (Figure 1f and Supplementary Figure S3c). No differences in the flowering phenotype were observed following UV-B irradiation in the WT L*er* ecotype, either in the numbers of rosettes and cauline leaves at flowering or the number of days before bolting. In *uvr8-2* plants, the number of days before bolting increased slightly, but the numbers of rosette and cauline leaves at flowering did not change after UV-B irradiation. When the independent experiments were repeated, the effects of UV-B irradiation on the flowering phenotype were similar throughout both tests, with no significant difference in the days before bolting in the second test for *uvr8-2* (Supplementary Table S2). Therefore, UV-B irradiation did not alter the flowering phenotype under the tested experimental conditions, regardless of UV-B irradiation intensity under LD conditions.

3.2 | UV-B Irradiation Delays Flowering of WT Under the SD Photoperiod

Next, we investigated the effect of UV-B irradiation on *Arabidopsis* flowering during an SD photoperiod. Representative images of 37 DAS *Arabidopsis* exposed to 0.1 $W m^{-2} s^{-1}$ for 7.5 h per day show that UV-B irradiation delayed bolting in both the WT L*er* ecotype and *uvr8–2* (Figure 2a). UV-B irradiation increased the number of rosette leaves at flowering in the WT plants and decreased it in *uvr8-2* plants (Figure 2b). However, no significant difference was observed in the number of cauline leaves between WT and *uvr8-2* plants under UV-B irradiation. UV-B irradiation significantly increased the number of days before bolting in both the WT and *uvr8-2* plants. The average number of days before bolting in the WT was 32.9 days for the nonirradiated UV-B control and 39.8 days for the UV-B-irradiated control. In contrast, the average number of days before bolting in *uvr8-2* was 33.0 days for the nonirradiated control and 36.6 days for the UV-B-irradiated control. UV-B irradiation delayed the number of days before bolting in both the WT and *uvr8-2*, but the delay was significantly shorter in the latter. In addition, UV-B irradiation increased the fresh weight of the WT, but decreased it in *uvr8-2* (Figure 2c).

Similar trends were seen with 0.6 $W m^{-2} s^{-1}$ UV-B irradiation for 1 h per day, but unlike the 0.1 $W m^{-2} s^{-1}$ UV-B irradiation for 15.5 h per day, the number of cauline leaves increased with UV-B irradiation in WT and decreased in *uvr8-2* (Figure 2d). These results suggest that UV-B irradiation delays flowering in WT plants under the SD photoperiod. However, in *uvr8-2* plants, flowering was delayed by UV-B irradiation when the flowering phenotype was determined based on the number of days before bolting, but it was accelerated when the flowering phenotype was determined based on the number of rosette leaves.

3.3 | UV-B Irradiation Increases *FT* Expression in WT Under the LD Photoperiod

Under the LD photoperiod, some reports indicate that UV-B irradiation increases, whereas others indicate that it decreases *FT* expression (Dotto et al. 2018; Zioutopoulou et al. 2022). Therefore, we investigated the effects of UV-B irradiation on *FT* mRNA expression in rosette leaves in this study (Figure 3). In WT plants, UV-B irradiation at an intensity of 0.1 $W m^{-2} s^{-1}$ for 15.5 h per day significantly increased *FT* expression level under the LD photoperiod at 10 DAS, 2 days after the start of UV-B irradiation (Figure 3a). Expression levels of *FT* were also increased by UV-B irradiation in WT plants at 13 and 17 DAS. Similarly, UV-B irradiation at an intensity of 0.1 $W m^{-2} s^{-1}$ for 1 h per day and 0.6 $W m^{-2} s^{-1}$ for 1 h per day also significantly increased *FT* levels in WT plants. In contrast, *uvr8-2* plants showed no increase in *FT* levels. Under the SD photoperiod, UV-B irradiation did not promote the expression of *FT*, even in WT plants (Figure 3b). In contrast, *FT* levels in *uvr8-2* plants increased but were 2 orders of magnitude lower than those under the LD photoperiod. Similarly, exposure to 0.6 $W m^{-2} s^{-1}$ UV-B for 1 h per day increased the *FT* levels in *uvr8-2* plants, but the difference was not significant. These results suggest that UV-B irradiation increases *FT* expression in a UVR8-dependent manner under LD conditions.

3.4 | UV-B Irradiation Does Not Increase *CO* Expression

Zioutopoulou et al. (2022) reported that UV-B irradiation increases the expression of *CO*, a crucial regulator of *FT* expression under the LD photoperiod. To investigate whether UV-B irradiation conditions used in this study affected the expression of *CO*, expression level was measured using quantitative RT-PCR. *Arabidopsis* plants were assessed at 10, 13, and 17 DAS under the LD photoperiods with UV-B irradiation at 0.1 $W m^{-2} s^{-1}$ for 15.5 h per day. These days indicate 2, 5, and 9 days after the start of UV-B irradiation. *CO* expression levels were not affected by UV-B irradiation under the LD photoperiod on any of the tested days (Figure 4a). We further examined *CO* expression under the SD photoperiod with UV-B irradiation at 0.1 $W m^{-2} s^{-1}$ for 7.5 h per day. UV-B irradiation under the SD photoperiod did not upregulate *CO* expression (Figure 4b). These results suggest that the increase in *FT* expression caused by UV-B irradiation is not caused by an increase in *CO* expression.

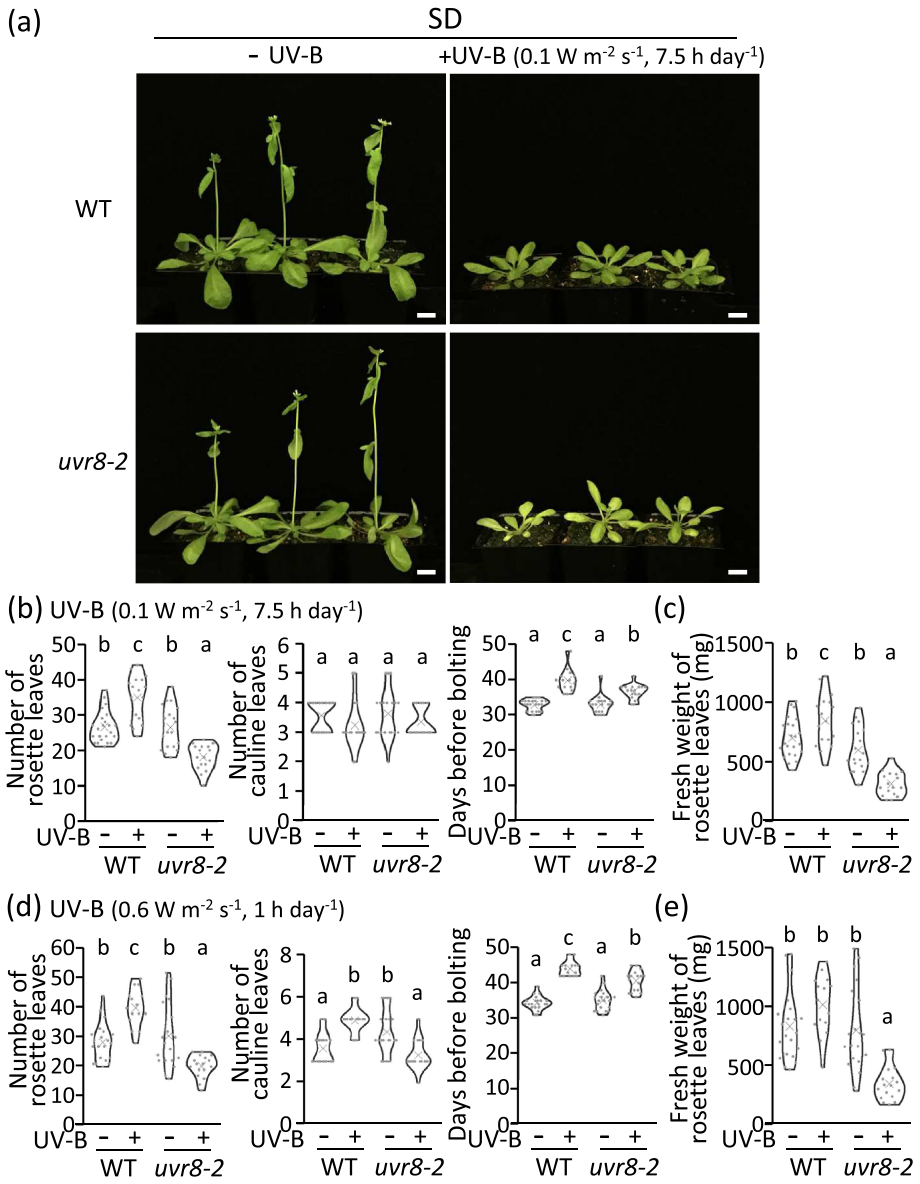

FIGURE 2 | UV-B irradiation delays the flowering of WT plants under the short-day (SD) photoperiod. (a) Representative images of 37-day-old WT and *uvr8-2* plants grown under the SD photoperiod without (–) or with (+) UV-B irradiation at an intensity of 0.1 W m^{-2} s^{-1}. The irradiation time was 7.5 h per day. Bars = 1 cm. (b–e) WT and *uvr8-2* plants were grown under the SD photoperiod without (–) or with (+) UV-B irradiation at an intensity of 0.1 W m^{-2} s^{-1} for 7.5 h per day (b, c), or 0.6 W m^{-2} s^{-1} for 1 h per day (d, e). Flowering time was measured by the numbers of rosette and cauline leaves when the first flower opened and the number of days before bolting (b, d). Fresh weight of rosette leaves was measured when the first flower opened (c, e). Data indicate the values of 15–23 plants (n = 15–23) (b, c) or 15–21 plants (n = 15–21) (d, e). Letters on the top of bars indicate the statistical significance determined via Tukey's multiple-comparison test ($p < 0.05$).

3.5 | UV-B Irradiation Does Not Alter the Expression Levels of Floral Meristem Identity Genes Under the LD Photoperiod

Under the LD photoperiod, UV-B irradiation increased *FT* expression (Figure 3a) but did not accelerate flowering (Figure 1). FT proteins cause flowering by translocating to SAM and inducing the expression of floral meristem identity genes. Therefore, we investigated whether UV-B irradiation affects the expression levels of floral meristem identity genes in SAM. *Arabidopsis* plants were analyzed at 10 and 13 DAS under the LD photoperiod with UV-B irradiation at 0.1 W m^{-2} s^{-1} for 15.5 h per day. The shoot apex, excluding the rosette leaves but containing SAM, was harvested from *Arabidopsis* aboveground parts, and RNA was extracted. We determined the expression levels of apetala 1 (*AP1*), leafy (*LFY*), fruitful (*FUL*), and suppressor of overexpression of CONSTANS 1 (*SOC1*) via quantitative RT-PCR (Figure 5). Gene expression levels were significantly higher at 13 DAS than at 10 DAS. UV-B irradiation at 13 DAS significantly increased *FUL* levels but did not significantly affect the levels of other genes. In *uvr8-2* plants, no significant changes were observed in any gene expression after UV-B irradiation. These findings suggest that UV-B irradiation does not increase expression of floral meristem identity genes under the LD photoperiod, despite increasing *FT* expression in WT plants.

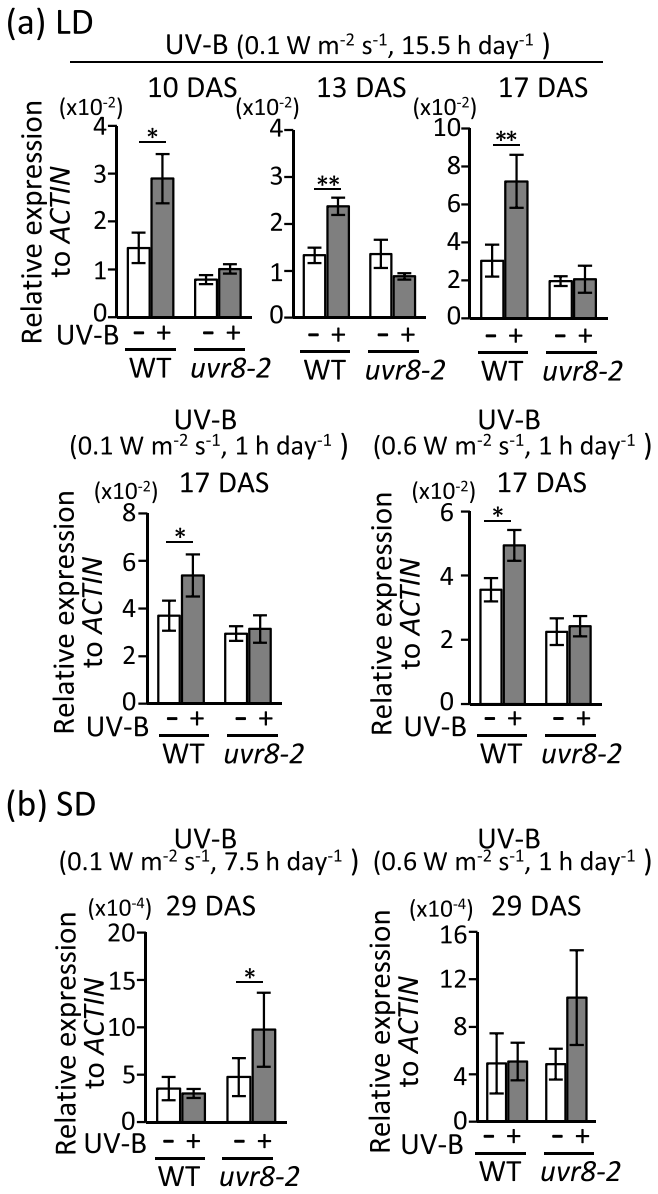

FIGURE 3 | UV-B irradiation increases *FT* expression in WT plants under the LD photoperiod. (a) Under the LD photoperiod, WT and *uvr8-2* plants were grown without (–) or with (+) UV-B irradiation at 0.1 W m^{-2} s^{-1} for 15.5 h per day, 0.1 W m^{-2} s^{-1} for 1 h per day, or 0.6 W m^{-2} s^{-1} for 1 h per day. Rosette leaves were collected from 10, 13, or 17 days after sowing (DAS) plants. Total RNA was extracted, and quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed using gene-specific primers for *FT*. For 10 and 13 DAS samples, the experiment was repeated thrice with three plants per biological repeat ($n = 3$). For 17 DAS samples, the experiment was repeated six times using different plants ($n = 6$). The data are relative to those of *ACTIN* and represented as the mean ± standard deviation. (b) Under the SD photoperiod, WT and *uvr8-2* plants were grown without (–) or with (+) UV-B irradiation at 0.1 W m^{-2} s^{-1} for 7.5 h per day or 0.6 W m^{-2} s^{-1} for 1 h per day. RT-PCR was performed on 29 DAS plants using gene-specific primers for *FT*. The experiment was repeated six times using different plants ($n = 6$). The data are relative to those of *ACTIN* and represented as the mean ± standard deviation. Asterisks indicate the statistically significant differences determined via Student's *t*-tests. *$p < 0.05$ and **$p < 0.01$.

4 | Discussion

It has been reported that UV-B irradiation causes a variety of flowering phenotypes in *Arabidopsis*, including unchanged, delayed, and accelerated flowering, under LD photoperiods (Arongaus et al. 2018; Dotto et al. 2018; Zioutopoulou et al. 2022). However, the mechanisms underlying this variation remain unknown. We hypothesized that the flowering phenotype would be affected by the intensity of UV-B irradiation, which was suppressed when the intensity of UV-B irradiation was high and accelerated when the intensity was low. However, in our experiment, UV-B irradiation did not result in accelerated flowering, even at a lower intensity than that used by Zioutopoulou et al. (2022). These results suggest that the intensity of UV-B alone does not determine the flowering phenotype.

The transcription factor CO, which regulates *FT* expression, is known to be stabilized by blue light via cryptochrome 1 (CRY1) and cryptochrome 2 (CRY2) photoreceptors (Valverde et al. 2004). It is also known that this reaction is regulated by COP1/SPA (Jang et al. 2008). UV-B irradiation produces active UVR8 monomers that bind COP1/SPA (Favory et al. 2009; Rizzini et al. 2011; Cloix et al. 2012). Therefore, UVR8 activation by UV-B irradiation may suppress COP-1/SPA-dependent CO degradation. The stabilization of CO protein through UVR8 and COP1/SPA may lead to an enhancement in *FT* expression by UV-B irradiation under LD photoperiod. Under the SD photoperiod, it has been reported that the compact chromatin structure of the *FT* promoter region prevents CO binding (Bao et al. 2019). Furthermore, Chen et al. (2024) discovered that the function of CO is suppressed by RUP2 under SD photoperiods. Through these pathways, even if the level of CO protein increases during the SD photoperiod, the expression level of *FT* may not increase by UV-B irradiation.

UV-B irradiation significantly increased *FT* levels in WT plants by 2.0-fold at 10 DAS and 1.8-fold at 13 DAS compared with those in the nonirradiated control in a UVR8-dependent manner (Figure 3a). However, in WT plants, expression levels of floral meristem identity genes in SAM were not increased by UV-B irradiation, except for *FUL* (Figure 5). Expression levels of floral meristem identity genes increased significantly from 10 to 13 DAS with and without UV-B irradiation, even in *uvr8-2* where *FT* was not increased by UV-B irradiation (Figure 5). These results suggest that UV-B irradiation results in increased *FT* expression, but this increase may not be predominant in inducing the expression levels of floral meristem identity genes. Zioutopoulou et al. (2022) reported that *FT* was induced approximately 6-fold in the L*er* ecotype and 2.5-fold in the Col-0 ecotype at 12 DAS and that flowering was accelerated in both cases. Therefore, if *FT* is induced at a higher level than in this experiment, it is possible that flowering will be induced even under UV-B irradiation. In our experiment, the intensity of visible light was set at approximately 100 μmol m^{-2} s^{-1}, but in the experiment by Zioutopoulou et al. (2022), the intensity of visible light was weaker at 50 μmol m^{-2} s^{-1}. It is well known that the expression of *FT* is regulated by visible light (Yanovsky and Kay 2002). Therefore,

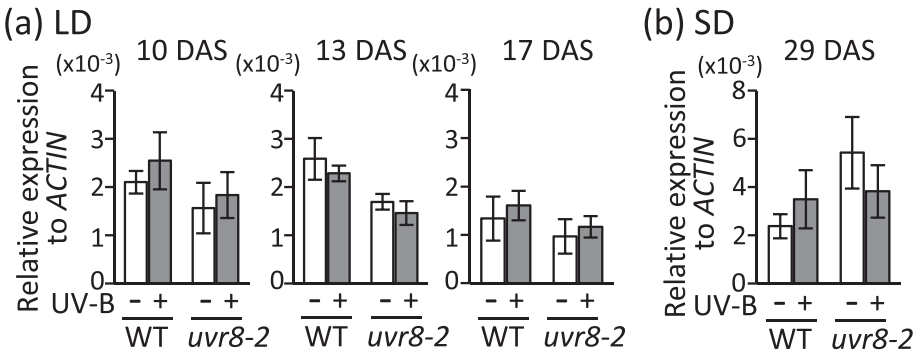

FIGURE 4 | UV-B irradiation does not increase the expression of *CO*. (a, b) WT and *uvr8-2* plants were grown without (−) or with (+) UV-B irradiation at an intensity of 0.1 W m^{-2} s^{-1} for 15.5 h per day under LD photoperiods (a) or 0.1 W m^{-2} s^{-1} for 7.5 h per day under SD photoperiods (b). Total RNA was extracted from rosette leaves. Quantitative RT-PCR was performed using gene-specific primers for *CO*. For 10 and 13 DAS samples, the experiment was repeated thrice with three plants per biological repeat ($n = 3$). For 17 and 29 DAS samples, the experiment was repeated six times using different plants ($n = 6$). Data are relative to those of *ACTIN* and represented as the mean ± standard deviation.

the effect of UV-B radiation on *FT* expression may fluctuate depending on the intensity of concurrently exposed visible light. In addition, it has been reported that UV-B irradiation changes the pace of the circadian clock in a UVR8-dependent manner (Fehér et al. 2011). Because *FT* expression is influenced by the circadian clock (Takagi et al. 2023), it is possible that UV-B irradiation affects the daily expression pattern of *FT* in response to changes in the circadian clock, but the overall expression level remained unchanged. In this experiment, *FT* was only detected at ZT15-16, irrespective of the presence of UV-B irradiation. In the future, it will be essential to examine the daily fluctuations in *FT* expression levels and assess the impact of UV-B irradiation on the total amount of *FT*.

The study by Dotto et al. (2018) reported that UV-B irradiation decreases the expression levels of floral meristem identity genes in SAM and increases those of micro-RNA 156 (*miR156*) in a UVR8-dependent manner under both the LD and SD photoperiods. It is possible that a comparable mechanism is also functioning in our experiment. Therefore, if *miR156* increases in the SAM by UV-B irradiation, it is possible that the expression of floral meristem identity genes will be suppressed even if *FT* expression increases. The current analysis (Figure 2; Supplementary Figure S5), along with previous studies conducted under the SD photoperiod (Arongaus et al. 2018; Dotto et al. 2018), revealed that exposure to UV-B radiation impairs the flowering process. It has been demonstrated that having high levels of *FT* expression is not essential to flowering under SD photoperiods (Jang et al. 2009), and the experiment showed that *FT* expression levels remained low (Figure 3b). Thus, in the case of SD photoperiods, where *FT* does not contribute to the flowering process, flowering inhibition by *miR156* may be the predominant function under UV-B irradiation. If the increased expression of *FT* by UV-B irradiation is more effective than the suppression of floral meristem identity genes in SAM, UV-B irradiation may successfully accelerate flowering. However, further investigation of *FT* mutants is necessary to determine whether changes in *FT* expression are the main factors causing UV-B-induced phenotypic differences in plants.

In this study, the number of days before bolting in *uvr8-2* plants increased under the SD photoperiod, but the number of rosette leaves during flowering decreased compared with the UV-B non-irradiated control (Figures 2). Flowering was delayed when measured in the days before bolting but accelerated when measured in rosette leaves. According to Pouteau and Albertini (2009), the timing of bolting is governed not only by the onset of the vegetative-to-reproductive phase change but also by factors such as the production of sufficient biomass for optimal seed yield. When comparing the flowering times in samples with varying growth rates, the number of rosette leaves was used rather than the number of days before bolting (Posé et al. 2013; Chen and Ludewig 2018). It is known that the *uvr8* mutant is inhibited in growth by UV-B irradiation (Kliebenstein et al. 2002; Brown et al. 2005). Therefore, because UV-B exposure affects the growth rate of *uvr8* mutants, the number of rosette leaves can be used to predict flowering time. Based on the number of rosette leaves, *uvr8-2* plants under the SD photoperiod exhibited accelerated flowering after UV-B irradiation (Figure 2b,d). UV-B irradiation under the SD photoperiod increased *FT* expression level significantly in *uvr8-2*; however, the level was 2 orders of magnitude lower than that under the LD photoperiod (Figure 3). Consequently, the mechanism by which UV-B promotes flowering in *uvr8-2* remains unclear. UVR8 is known to be involved in the expression of genes for the biosynthesis of UV-absorbing compounds and CPD photolyase (Brown et al. 2005); therefore, UVR8 deficiency may increase UV-B-induced DNA damage. *Arabidopsis* mutants of ataxia telangiectasia mutated, which are activated by DNA double-strand breaks, accumulate more DNA damage and flower earlier than the WT (Su et al. 2017; Zhang et al. 2020). Therefore, the accumulated DNA damage caused by UV-B irradiation may accelerate flowering, which is an important issue for future research.

In conclusion, this study showed that despite UV-B irradiation increasing *FT* levels, the expression levels of floral meristem identity genes in SAM remain unchanged, suggesting that the primary effect of UV-B on flowering is a suppressive one. UVR8 is known to be activated by weak UV-B radiation that does not cause damage, but it induces a gene that enhances tolerance to UV-B damage (Brosché and Strid 2002; Brown and Jenkins 2008). Here, plants exposed to weak UV-B radiation may exhibit delayed flowering, allowing them to prepare for subsequent exposure to high levels of UV-B radiation. Delaying

flowering to allow recovery from UV-B damage may be beneficial to ensure sufficient energy supply for seed production. Flowering might be induced by UV-B irradiation only when the flowering-promoting effect is greater than the flowering-inhibiting effect. Further analysis is needed to fully understand how the balance between UV-B induced flowering promotion and flowering inhibition is controlled.

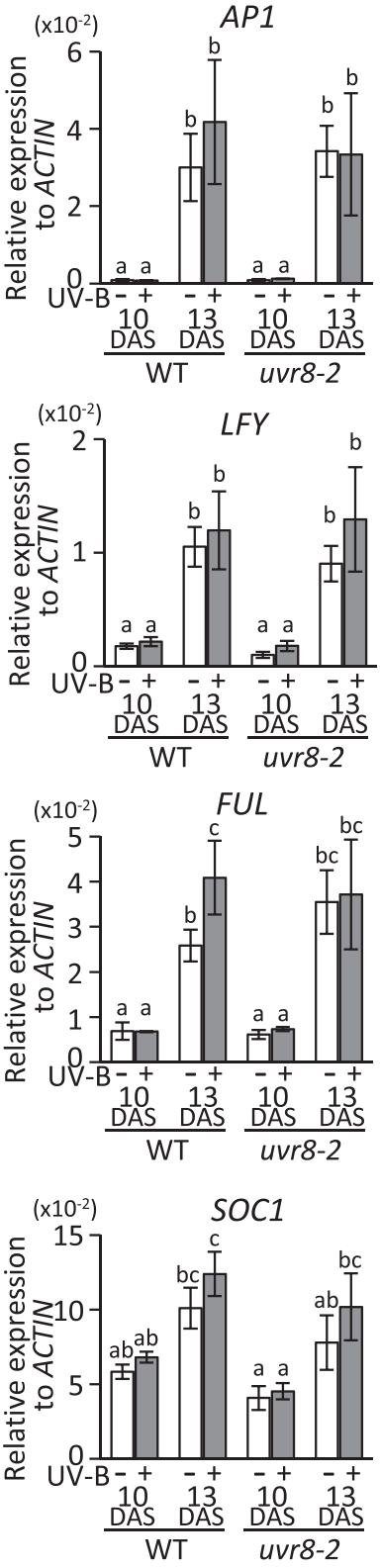

FIGURE 5 | Expression levels of floral meristem identity genes are not altered by UV-B irradiation under the LD photoperiod. (a) Under the LD photoperiod, WT and *uvr8-2* plants were grown without (–) or with (+) UV-B irradiation at 0.1 W m^{-2} s^{-1} for 15.5 h per day. Shoot apices were collected from 10 and 13 DAS plants. Total RNA was extracted, and quantitative RT-PCR was performed using gene-specific primers for apetala 1 (*AP1*), leafy (*LFY*), fruitful (*FUL*), and suppressor of overexpression of CONSTANS 1 (*SOC1*). Data are relative to those of *ACTIN* and represented as the mean ± standard deviation of three biological repeats ($n = 3$). Letters on the top of bars indicate the statistical significance determined via Tukey's multiple-comparison test ($p < 0.05$).

Author Contributions

M.T. designed the experiments. A.T. and M.T. conducted the experiments and analyzed the data. Y.T. provided insights that led to the conception of this study. J.H. contributed to the acquisition of experimental plant lines and supervised the project. M.T. wrote the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.